

WHAT IS CLAIMED IS:

1. A method for making a nucleic acid molecule comprising
 - (a) mixing a nucleic acid template with (i) one or more polypeptides having polymerase activity and/or reverse transcriptase activity and (ii) a primer-adapter nucleic acid molecule; and
 - (b) incubating said mixture under conditions sufficient to make a first nucleic acid molecule complementary to all or a portion of said template, wherein said primer-adapter nucleic acid molecule comprises one or more ligands and one or more cleavage sites.
2. The method of claim 1, wherein said first nucleic acid molecule comprises said primer-adapter nucleic acid molecule.
3. The method of claim 1, wherein said template is RNA or DNA.
4. The method of claim 3, wherein said RNA is a mRNA or a polyA+ RNA molecule.
5. The method of claim 1, wherein said first nucleic acid molecule is RNA or DNA.
6. The method of claim 1, wherein said polypeptide is selected from the group consisting of a Moloney Leukemia Virus (M-MLV) reverse transcriptase, a Rous Sarcoma Virus (RSV) reverse transcriptase, an Avian Myeloblastosis Virus (AMV) reverse transcriptase, a *Tne* DNA polymerase, a *Tma* DNA polymerase, a *Taq* DNA polymerase, a *Tth* DNA polymerase, a *Tli* or VENT™ DNA polymerase, a *Pfu* or DEEPVENT™ DNA polymerase, a *Pwo* DNA polymerase, a *Bst* DNA polymerase, a *Sac* DNA polymerase, a *Tac* DNA

(xxix) a transferrin receptor; (xxx) Fe^{+++} ; (xxxi) polymyxin B or endotoxin-neutralizing protein (ENP); (xxxii) an enzyme-specific substrate; (xxxiii) protein A, protein G, a cell-surface Fc receptor or an antibody-specific antigen; and (xxxiv) avidin and streptavidin.

5 11. The method of claim 1, wherein said cleavage site is a restriction endonuclease cleavage site or an endonuclease cleavage site.

10 ~~12.~~ The method of claim 2, ~~said method further comprising incubating~~ said first nucleic acid molecule under conditions sufficient to make a second nucleic acid molecule complementary to all or a portion of said first nucleic acid molecule.

 13. The method of claim 12, wherein said second nucleic acid molecule is a RNA or a DNA molecule.

 14. The method of claim 12, wherein said first and said second nucleic acid molecules form a double-stranded nucleic acid molecule.

15 15. The method of claim 14, wherein said double-stranded nucleic acid molecule is a double-stranded cDNA molecule.

~~16.~~ The method of claim ~~12~~, wherein said incubation step comprises mixing said first nucleic acid molecule with a DNA polymerase, one or more nucleotides and one or more primers.

20 ~~17.~~ The method of claim ~~16~~, wherein said primers are primer-adapters which comprise one or more ligands and one or more cleavage sites.

000764 0500
000764 0500
000764 0500

7
18. The method of claim 2, said method further comprising binding one or more of said ligands to one or more haptens thereby forming a nucleic acid-ligand-hapten complex.

8
19. The method of claim 12, said method further comprising binding one or more of said ligands to one or more haptens thereby forming a nucleic acid-ligand-hapten complex.

20. The method of claim 18 or claim 19, said method further comprising isolating said nucleic acid molecule from said complex by cleavage of one or more of said cleavage sites.

21. The method of claim 20, wherein said nucleic acid molecule is a double-stranded or a single-stranded nucleic acid molecule.

10
22. The method of claim 18 or claim 19, wherein said one or more haptens are bound to a solid support.

23. The method of claim 22, wherein said solid support is selected from the group consisting of nitrocellulose, diazocellulose, glass, polystyrene, polyvinylchloride, polypropylene, polyethylene, dextran, Sepharose, agar, starch, nylon, a latex bead, a magnetic bead, a paramagnetic bead, a superparamagnetic bead and a microtitre plate.

24. The method of claim 18 or claim 19, wherein said one or more haptens are selected from the group consisting of (i) avidin and streptavidin; (ii) protein A, protein G, a cell-surface Fc receptor or an antibody-specific antigen; (iii) an enzyme-specific substrate; (iv) polymyxin B or endotoxin-neutralizing protein (ENP); (v) Fe^{+++} ; (vi) a transferrin receptor; (vii) an insulin

receptor; (viii) a cytokine (*e.g.*, growth factor, interleukin or colony-stimulating factor) receptor; (ix) CD4; (x) spectrin or fodrin; (xi) ICAM-1 or ICAM-2; (xii) C3bi, fibrinogen or Factor X; (xiii) ankyrin; (xiv) integrins $\alpha_1\beta_1$, $\alpha_2\beta_1$, $\alpha_3\beta_1$, $\alpha_6\beta_1$, $\alpha_7\beta_1$ and $\alpha_6\beta_5$; (xv) integrins $\alpha_1\beta_1$, $\alpha_2\beta_1$, $\alpha_3\beta_1$ and $\alpha_v\beta_3$; (xvi) integrins $\alpha_3\beta_1$, $\alpha_4\beta_1$, $\alpha_4\beta_7$, $\alpha_5\beta_1$, $\alpha_v\beta_1$, $\alpha_{IIB}\beta_3$, $\alpha_v\beta_3$ and $\alpha_v\beta_6$; (xvii) integrins $\alpha_v\beta_1$ and $\alpha_v\beta_3$; (xviii) vitronectin; ~~(xix) fibronectin~~; (xx) collagen; (xxi) laminin; (xxii) glycophorin; (xxiii) Mac-1; (xxiv) LFA-1; (xxv) β -actin; (xxvi) gp120; (xxvii) cytokines (growth factors, interleukins or colony-stimulating factors); (xxviii) insulin; (xxix) ferrotransferrin; (xxx) apotransferrin; (xxxi) lipopolysaccharide; (xxxii) an enzyme; (xxxiii) an antibody; and (xxxiv) biotin.

25. The method of ~~claim 2~~, said method further comprising amplifying said first nucleic acid molecule.

26. The method of claim 25, wherein said amplification is accomplished by a method comprising incubating said first nucleic acid molecule with a DNA polymerase, one or more nucleotides and one or more primers.

27. The method of claim 26, wherein said primers are primer-adapters.

28. The method of ~~claim 12~~, said method further comprising amplifying said first and second nucleic acid molecules.

29. The method of claim 28, wherein said amplification is accomplished by a method comprising

(a) contacting said first nucleic acid molecule with a first primer-adapter which is complementary to a portion of said first nucleic acid molecule, and a second nucleic acid molecule with a second primer-adapter which is

complementary to a portion of said second nucleic acid molecule, with a polypeptide having polymerase and/or reverse transcriptase activity;

(b) incubating said mixture under conditions sufficient to form a third nucleic acid molecule complementary to all or a portion of said first nucleic acid molecule and a fourth nucleic acid molecule complementary to all or a portion of said second nucleic acid molecule;

(c) denaturing said first and third and said second and fourth nucleic acid molecules; and

(d) repeating steps (a) through (c) one or more times.

30. The method of claim 29, wherein said first primer-adapter or said second primer adapter is replaced with an oligonucleotide primer.

31. The method of claim 29, said method further comprising binding one or more of said ligands to one or more haptens, thereby forming a nucleic acid-ligand-hapten complex with said amplified nucleic acid.

32. The method of claim 31, wherein said method further comprises isolating said nucleic acid from said complex by cleaving one or more of said cleavage sites.

33. A nucleic acid molecule comprising one or more primer-adapter molecules, wherein said primer-adapter molecule comprises one or more ligands and one or more cleavage sites.

34. The nucleic acid molecule of claim 33, wherein said one or more cleavage sites allows removal of said one or more ligands from said nucleic acid molecule.

RECEIVED SEP 20 1990

15
Sub B₂

polymerase, a *Tfi/Tub* DNA polymerase, a *Tru* DNA polymerase, a DYNAZYME™ DNA polymerase, an *Mth* DNA polymerase, a Rous Associated Virus (RAV) reverse transcriptase, a Myeloblastosis Associated Virus (MAV) reverse transcriptase, a Human Immunodeficiency Virus (HIV) reverse transcriptase, a retroviral reverse transcriptase, a retrotransposon reverse transcriptase, a hepatitis B virus reverse transcriptase, a cauliflower mosaic virus reverse transcriptase, a bacterial reverse transcriptase and mutants, variants and derivatives thereof.

7. The method of claim 4, wherein said first nucleic acid molecule is a cDNA molecule.

8. The method of claim 1, wherein said cleavage site allows removal of at least one of said ligands from said primer-adaptor nucleic acid molecule.

9. The method of claim 2, wherein said cleavage site allows removal of at least one of said ligands from said first nucleic acid molecule.

10. The method of claim 1, wherein said ligand molecule is selected from the group consisting of (i) biotin; (ii) an antibody; (iii) an enzyme; (iv) lipopolysaccharide; (v) apotransferrin; (vi) ferrotransferrin; (vii) insulin; (viii) cytokines (growth factors, interleukins or colony-stimulating factors); (ix) gp120; (x) β -actin; (xi) LFA-1; (xii) Mac-1; (xiii) glycophorin; (xiv) laminin; (xv) collagen; (xvi) fibronectin; (xvii) vitronectin; (xviii) integrins $\alpha_v\beta_1$ and $\alpha_v\beta_3$; (xix) integrins $\alpha_3\beta_1$, $\alpha_4\beta_1$, $\alpha_4\beta_7$, $\alpha_5\beta_1$, $\alpha_v\beta_1$, $\alpha_{IIB}\beta_3$, $\alpha_v\beta_3$ and $\alpha_v\beta_6$; (xx) integrins $\alpha_1\beta_1$, $\alpha_2\beta_1$, $\alpha_3\beta_1$ and $\alpha_v\beta_3$; (xxi) integrins $\alpha_1\beta_1$, $\alpha_2\beta_1$, $\alpha_3\beta_1$, $\alpha_6\beta_1$, $\alpha_7\beta_1$ and $\alpha_6\beta_5$; (xxii) ankyrin; (xxiii) C3bi, fibrinogen or Factor X; (xxiv) ICAM-1 or ICAM-2; (xxv) spectrin or fodrin; (xxvi) CD4; (xxvii) a cytokine (e.g., growth factor, interleukin or colony-stimulating factor) receptor; (xxviii) an insulin receptor;

35. The nucleic acid molecule of claim 33, wherein said ligand is bound to one or more haptens.

36. The nucleic acid molecule of claim 35, wherein said one or more haptens is bound to a solid support.

37. The nucleic acid molecule of claim 33, wherein said cleavage site is a restriction endonuclease site or an endonuclease cleavage site.

38. The nucleic acid molecule of claim 33, wherein said nucleic acid molecule is double-stranded or single-stranded.

39. The nucleic acid molecule of claim 38, wherein said nucleic acid molecule is a DNA molecule, a RNA molecule, or a DNA/RNA hybrid molecule.

40. A nucleic acid molecule produced by the method of claim 1 or claim 12.

41. A kit for the production of a nucleic acid molecule comprising one or more containers, wherein a first container comprises a primer-adaptor molecule comprising one or more ligands and one or more cleavage sites.

42. The kit of claim 41, further comprising one or more additional containers comprising one or more polypeptides having polymerase and/or reverse transcriptase activity.

43. The kit of claim 41, further comprising one or more additional containers comprising a solid support which comprises one or more haptens which specifically recognize and are capable of binding said ligand.

0004160313
35250-3752000

44. A method for producing a cDNA molecule, said method comprising

(a) mixing an mRNA template with a polypeptide having reverse transcriptase activity and a primer-adapter nucleic acid molecule, said primer-adapter molecule comprising one or more ligands and one or more cleavage sites;

(b) incubating said mixture under conditions sufficient to make a first DNA molecule complementary to all or a portion of said template, thereby forming a DNA-primer-adapter molecule;

(c) binding said DNA-primer-adapter molecule to a solid support through a ligand-hapten interaction; and

(d) isolating said first DNA molecule from said solid support by cleaving said one or more cleavage sites.

45. The method of claim 44, said method further comprising making a second DNA molecule complementary to all or a portion of said first DNA molecule.

46. The method of claim 45, wherein said second DNA molecule is made before or after said isolation of said first DNA molecule.

47. The method of claim 46, wherein said second DNA molecule comprises a primer-adapter molecule.

48. The method of claim 44, wherein said method is used to prepare a cDNA library from a mRNA sample.

49. A method for producing a cDNA molecule, said method comprising

000415 0513
36250 373200

(b) incubating said first DNA molecule with a primer-adapter molecule, wherein said primer-adapter molecule comprises one or more ligands and one or more cleavage sites, under conditions sufficient to form a double-stranded DNA molecule comprising a primer-adapter molecule;

(d) isolating said double-stranded DNA molecule from said solid support by cleaving said one or more cleavage sites.

50. The method of claim 49, wherein said method is used to prepare a cDNA library from a mRNA sample.

51. A method for isolating a mRNA molecule, said method comprising

(a) mixing a RNA sample with a primer-adapter molecule which hybridizes to mRNA, wherein said primer-adapter molecule comprises one or more ligands and one or more cleavage sites, thereby forming a mRNA-primer-adapter molecule;

(b) binding said mRNA-primer/adaptor molecule to a solid support through a ligand-hapten interaction; and

(c) isolating said mRNA molecule from said solid support by cleaving said one or more cleavage sites;

52. The method of claim 51, wherein said primer-adaptor molecule comprises oligo(dT).

53. A method for isolating one or more desired nucleic acid molecules from a population of nucleic acid molecules comprising

(a) mixing said population of nucleic acid molecules with one or more target-specific primer-adapter molecules;

(b) incubating said mixture under conditions sufficient to bind said primer-adapter molecules to said desired nucleic acid molecules; and

(c) isolating one or more of said desired nucleic acid molecules.

Add D²

ADD
E1

09075415 051298